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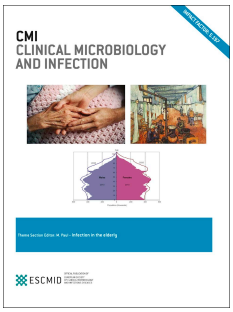

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Aetiology of Lower Respiratory Tract Infection in Adults in Primary Care: A prospective Study in 11 European Countries

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ABSTRACT

Objectives: To describe the role of bacteria (including bacterial resistance), viruses (including those recently described), and mixed bacterial-viral infections in adults presenting to primary care with lower respiratory tract infection (LRTI).

Methods: We enrolled 3104 adults with LRTI, 141 (4.5%) of whom had community-acquired pneumonia (CAP), and 2985 matched controls in a prospective study in 16 primary care networks in Europe, and followed patients up at 28-35 days. We detected *S. pneumoniae* and *H. influenzae* and assessed susceptibility, atypical bacteria and viruses.

Results: A potential pathogen was detected in 1844 (59%) (in 350 (11%) bacterial pathogens only, in 1190 (38%) viral pathogens only, and in 304 (10%) both bacterial and viral pathogens). The most common bacterial pathogens isolated were *S. pneumoniae* (5.5% overall, 9.2% in CAP patients) and *H. influenzae* (5.4% overall, 14.2% in CAP patients). <1% of *S. pneumoniae* were highly resistant to penicillin and 12.6% of *H. influenzae* were beta-lactamase positive. The most common viral pathogens detected were human rhinovirus (HRV; 20.1%), influenza viruses (FLU; 9.9%), and human coronavirus (HCoV; 7.4%). FLU, human parainfluenzaviruses and human respiratory syncytial virus as well as HRV, HCoV, human metapneumovirus were detected significantly more frequently in LRTI patients than in controls.

Conclusions: A bacterial pathogen is identified in approximately one in five adult patients with LRTI in primary care, and a viral pathogen in just under half, with mixed infections in one in ten. Penicillin resistant pneumococci and beta-lactamase producing *H. influenzae* are uncommon. These new findings support a restrictive approach to antibiotic prescribing for LRTI and the use of first-line, narrow spectrum agents in primary care.

INTRODUCTION

Community-acquired lower respiratory tract infection (LRTI) is one of the commonest reasons for consulting in primary care and accounts for considerable antibiotic use and health care costs. It is neither feasible nor cost-efficient to identify microbial aetiology in most patients who present with LRTI in primary care because of sampling challenges, limited access diagnostics, and the limited clinical utility of receiving a result after empirical treatment decision has been made (1). Consequently, little is known about the aetiology of LRTI in everyday primary care. In addition, detecting pathogens in both symptomatic patients and contemporaneous controls to distinguish between asymptomatic carriage and the presence of agents causing symptoms has rarely been done. Nevertheless, despite limited knowledge of the proportion of patients that have an identifiable bacterial aetiology and the sensitivities of these pathogens, and evidence of limited or no clinical benefit from antibiotic treatment, more than half of patients presenting to primary care with LRTI/acute cough in Europe are prescribed antibiotics (2-4). This contributes to the selection of antimicrobial resistant bacteria (5). Improved knowledge of likely pathogens (at the point of care) and the likely susceptibility of bacterial pathogens, could help guide antibiotic prescribing decisions and thus help contain unnecessary antibiotic use and antimicrobial resistance. Furthermore, such information could support public health policy on prevention of respiratory illness including vaccination.

Therefore, our primary objective was to describe the viral and bacterial aetiology in adult patients presenting to primary care with lrti and in those with community-acquired pneumonia (cap). our secondary objectives were to describe the presence of resistance in bacterial infections and of mixed viral-bacterial infections.

MATERIALS AND METHODS

Study design and patients

The study was part of the European Union FP6 funded Network of Excellence GRACE (Genomics to combat Resistance against Antibiotics in Community-acquired LRTI in Europe Network of Excellence; www.grace-lrti.org). We recruited patients between October 2007 and April 2010 in 16 primary care networks (PCNs) that had a track record of conducting research based in 11 European countries: Antwerp and Ghent (Belgium); Barcelona and Mataro (Spain); Bialystok, Lodz and Szczecin (Poland); Bratislava (Slovakia); Cardiff and Southampton (UK); Jesenice (Slovenia); Jönköping (Sweden); Milan (Italy); Nice (France); Rotenburg (Germany), and Utrecht (The Netherlands).

Inclusion criteria for patients were: age ≥ 18 years, with an acute or worsened cough (≤28 days duration) as the main symptom, or any clinical presentation considered to be caused by LRTI by the general practitioner (GP) and consulting for the first time for this illness episode. Patients with presumed cough of non-infective origin, antibiotic consumption in the previous month, and any serious condition associated with an immunocompromised condition were excluded. For each patient, we planned to include a control patient matched for age, maximum 5 years of difference, and gender, consulting at the GP office for any other reason than acute respiratory illness within the same two-week period. The study was approved by the local ethics committees in all participating centres and by the competent authority in each country. Written informed consent was obtained from each patient and control prior to inclusion.

Sampling and Measurements

Symptomatic patients were assessed at first presentation (day 1) and between days 28-35. Chest radiographs were taken within one week after inclusion. Community-acquired

pneumonia (CAP) was considered present if the local radiologist reported lobar or bronchopneumonia; other diagnoses were categorised as 'pneumonia absent' (6).

All recruiting GPs received standardised sampling material and a protocol with detailed instructions on the sampling of the patients. Within 24 h of first presentation and inclusion, serum and EDTA blood, sputum, if available, and two nasopharyngeal flocked swabs (NPS; COPAN) were taken. At day 28-35, serum sampling and the two NPS were repeated. Controls were sampled for EDTA blood and two NPS at baseline. Sputum was not obtained from controls and controls were not followed up. Serum, EDTA and NPS were stored frozen in the local laboratories until regular shipment to the central laboratory (University Hospital Antwerp), where specimens were stored at -80°C until analysis.

Bacterial cultures for S. pneumoniae and H. influenzae

Sputum samples were examined in the local laboratories using direct microscopy to assess the quality (ratio of white blood cells/epithelial cells ≥1 as criterion for good quality), then Gram stained, cultured, and subsequently frozen at -80°C. *S. pneumoniae* and *H. influenzae* were identified using conventional biochemical tests and isolates were frozen in microbanks until shipped in batches to the central laboratory, where NPS were cultured for *S. pneumoniae* and/or *H. influenzae*. Their susceptibility was tested at the Karolinska Institute and the Oxford University, respectively, after frozen transport. Minimum inhibitory concentrations (MICs) of *S. pneumoniae* to penicillin G, erythromycin, clindamycin, tetracycline, and levofloxacin were determined. Isolates were classified as sensitive, indeterminate or resistant according to the EUCAST breakpoints for these species (www.eucast.org/antimicrobial-susceptibility-testing/breakpoints). *H. influenzae* isolates were tested for beta-lactamase production.

PCRs for Mycoplasma pneumoniae, Chlamydia pneumoniae, Bordetella pertussis, Legionella pneumophila and respiratory viruses

Nucleic acid from NPS was extracted with the NucliSens EasyMag (bioMérieux) in Antwerp after which aliquots were shipped and analysed in three collaborating laboratories for subsequent analysis with their in-house amplification and detection methods evaluated previously (7).

Serology for M. pneumoniae, C. pneumoniae and B. pertussis

For the detection of *M. pneumoniae*-specific and *C. pneumoniae* specific IgG or IgM antibodies, *M. pneumoniae* or *C. pneumoniae*–IgG and IgM-ELISA kits (Medac GmbH) were used according to the instructions of the manufacturer. IgG antibodies to *B. pertussis* toxin (PT; Virion/Serion) were analysed in a convalescent serum sample.

Diagnostic criteria

The isolation of *S. pneumoniae* and *H. influenzae,* and the identification of *L. pneumophila* or respiratory viruses by use of PCR in respiratory samples were considered to support an aetiological diagnosis. Infection with *M. pneumoniae* or *C. pneumoniae* was defined as: positive PCR in respiratory samples, the presence of IgM antibodies in the acute phase serum and/or convalescent phase sample, IgG seroconversion or a significant increase in IgG between acute and convalescent samples.

A patient was considered positive for an acute *B. pertussis* infection (infection in the last 6 months) if positive by PCR in a respiratory sample and/or the presence of an antibody titre to PT of ≥125 IU/ml in convalescent serum (day 28-35), demonstrated previously as a cut-off with high sensitivity and specificity (8, 9).

Statistical analysis

Generalized Estimating Equations were used to assess differences in the proportion of potential pathogens between LRTI patients' day 1 and day 28-35 samples, and between day 1 samples of LRTI patients and controls. The case-control design was applied to assess causality between viral pathogens and LRTI (CAP). Chi-square tests were used to assess differences in the proportion of specific viruses or bacteria between LRTI patients with and those without CAP. Student t-test was used to assess differences in age between LRTI patients with and those without specific viral or bacterial aetiology (IBM® SPSS® Statistics, Release 20.0.0). A P-value of <0.05 was considered to be statistically significant.

RESULTS

Patient characteristics and response

A total of 3104 adult LRTI patients were included by 294 GPs from October 2007 to April 2010, 1860 (60.0%) were female (Table 1). The mean age was 49.8 years (range 18 - 92 years) and 141 were diagnosed with CAP (4.5%); among elderly patients (>65 years n=628, 20.2%) 40 patients had a CAP (6.4%). We recruited a total of 2985 controls without symptoms of LRTI.

Day 1 NPS and blood samples were available from 3085 (99.4%) and 3054 (98.4%) LRTI patients, sputum samples in 2121 (68.3%). On day 28-35, 2673 patients (86.1%) were seen: in 2552 (95.5%) and 2575 (96.3%) of these, blood samples and NPSs, respectively, could be collected. Only controls who matched with patients according to all criteria (n= 2063) were further included to estimate causality.

Aetiology in LRTI and CAP in primary care

The proportion of patients with LRTI and CAP with an identified bacterial, viral, and mixed aetiology is presented in figure 1.

Bacterial aetiology and resistance in S. pneumoniae and H. influenzae.

A potential bacterial pathogen was found in 655 (21.1%) LRTI patients on day 1, significantly more often in patients with CAP compared to those without (Figure 1 and Table 2). *S. pneumoniae* and *H. influenza*e were significantly more prevalent in patients presenting with CAP. 9.2% of all 3104 patients and 10.6% of CAP patients were vaccinated against *S. pneumoniae*. Prevalence of pneumococci in these groups was 4.9% and 0%, respectively.

24/172 (14.0%) had a reduced susceptibility to penicillin G (1 isolate highly resistant, 23 (13.4%) intermediate resistance). Thirty-six (20.9%) isolates were less susceptible to erythromycin/clindamycin, 78 (45.3%) had a reduced susceptibility to tetracycline, and 3 (1,7%) were resistant to levofloxacin. 21/167 (12.6%) *H. influenzae* isolates produced beta-lactamases.

Viral aetiology

Any viral aetiology was identified in 1494 (48.1%) of LRTI patients, significantly less often in those with CAP compared to those without CAP (Figure 1 and Tables 2-3). The commonest viruses in our cohort of patients were HRV, FLU and HCoV. A respiratory virus was detected on day 28-35 in 336 patients (12.6%), as well as in 205 (9.9%) of matched controls. All respiratory viruses, except for HAdV, HBoV and WUPyV and KIPyV, were significantly more frequently detected in day 1 NPS of LRTI patients than in their day 28-35 NPS or in the NPS of their matched controls (Table 3). Apart from HAdV, virus prevalence did not differ significantly between patients with CAP or with LRTI.

23.6% of all LRTI patients and 29.1% of CAP patients were vaccinated against influenza. Prevalence of influenza in these groups was 5.3% and 4.9%, respectively.

Detection of atypical bacterial agents or viruses at follow-up within the same patient

Casewise analysis of atypical bacterial agents or viruses detected during illness compared to subsequent detection at follow-up is presented in Table 4. None of the patients who were initially PCR positive for *M. pneumoniae, B. pertussis*, FLU or HPIV 1-4 remained positive for these aetiologies at follow-up. Very few patients positive for HRV, HCoV, RSV, hMPV, polyomaviruses (WU+KI) and HBoV had the same pathogen detected at follow-up.

Mixed infections

Among all 3104 LRTI patients, a mixed bacterial, mixed viral or mixed bacterial-viral infection was detected in 51 (1.6%), 118 (3.8%) and 304 (9.8%) patients, respectively. The pathogens involved are described in more detail in the supplementary material.

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DISCUSSION

This is the only prospective, large international, case-control study using standardized sampling and comprehensive microbiological work up to provide accurate estimates of the prevalence of both bacterial and viral aetiology in patients consulting in primary care with LRTI. The overall microbiological yield was high, mainly due to the high prevalence of viruses. A potential bacterial pathogen was isolated in only one in five patients, and that antibiotic resistant pathogens were rare.

Comparison with literature

Previous studies have mainly studied more severely ill patients hospitalised with CAP rather than LRTI in primary care (1, 10-12), and few of those studies used comprehensive diagnostic methods, including PCR, to detect respiratory viruses (10, 12, 13). We identified a potential pathogen in about 60% of CAP patients. However, comparisons are difficult in that our study is unique in terms of study design, the broad inclusion criteria, the high numbers of patients sampled at baseline and follow up, the inclusion of matched controls, and comprehensive conventional and molecular microbiological diagnostics used.

Bacterial aetiology and resistance in CAP

The prevalence of *S. pneumoniae* and *H. influenzae* in our CAP subgroup were significantly higher than in the non-CAP patients, but lower in comparison to most previous studies. We do not consider that the implementation of pneumococcal vaccine influenced our findings because of the small number of CAP patients who had been vaccinated. However, only 5% of patients in the most comprehensive aetiological study of adult patients hospitalized with CAP in the US had pneumococcal pneumonia (14). High level penicillin resistance in pneumococci remains very low in all European countries in this setting, which supports the recommendation that if antibiotics are to be prescribed, amoxicillin should be the first-line

agent for LRTI (1). *M. pneumoniae* infections occur in epidemics every 4-5 years: we included patients in our study between two epidemic waves, possibly explaining the low *M. pneumoniae* prevalence observed (15, 16). This is also the first large European prospective study on the prevalence of pertussis in adults consulting primary care physicians for acute cough (17).

Importance of respiratory viruses, including newly detected viruses

We detected at least one respiratory viral pathogen in almost 50% of patients. NPS sampling may have yielded significantly more infected respiratory epithelial cells (18), with sensitive PCR based diagnostic techniques augmenting specifically for viruses.

FLU, HPIV 1-4 and RSV viruses are recognised causes of CAP in hospitalised patients and in the elderly[13), 22, 24.] Influenza vaccination resulted in lower prevalence of FLU in the elderly (data not shown). HRV, HCoV, and HMPV are rarely detected in CAP and other LRTI in outpatients.

HRV has been associated with outbreaks of severe respiratory disease, including CAP, in older people (19-21) and has been isolated in hospitalized patients with CAP (10), but a prevalence of 14.2% in CAP in outpatients is high and a novel finding. HCoV have recently been identified in small numbers of adults with severe pneumonia (10, 21), but is not routinely tested for in adult outpatients with CAP or LRTI. We may have underestimated the prevalence of HCoV as HKU-1 testing was not performed and HKU-1 is generally as prevalent as NL63 and OC43 (22). Infections due to HMPV are mainly described in long term care facilities (10). We found HMPV more prevalent in outpatients with CAP compared to those with other LRTIs, with even greater prevalence in CAP patients than RSV, and similar to the 3%-7% HMPV infection prevalence found in hospitalized adults (23). Although

numbers are small, HAdV was significantly more prevalent in CAP compared to other LRTI, a unique finding in immunocompetent outpatients. The high rates of viral detection in outpatients with LRTI and CAP suggests that comprehensive microbiological assessment is important to guide management and may explain the limited average benefit from antibiotic treatment in the placebo-controlled study we conducted in a large subset of patients included in the present analysis (2).

Our study is the first that compared the prevalence of respiratory viruses in symptomatic adults to that in matched controls without respiratory symptoms. FLU, HPIV 1-4 and RSV were never, or rarely, detected in controls or at follow-up in symptomatic patients. This strongly implicates these agents as causative pathogens. Similarly, the significantly lower prevalence of HRV, HMPV, and HCoV in patients at follow-up and in controls suggests that asymptomatic carriage of these viruses is uncommon in adults, and indicates that these viruses should also be regarded as causative agents in CAP (11),[14,](23).

For HRV, the rates of prolonged shedding (same genotype in 35%) versus reinfection (other genotype) in the GRACE study have been further investigated (24).

HBoV was detected in CAP and in <1% of LRTI patients at baseline, with similar findings among controls and at of follow up of patients. HBoV was identified in respiratory specimens from 1.5% hospitalized adults with no alternate viral aetiology, but controls were not included in that study (25). Since HBoV is often found in the presence of other pathogens in respiratory specimens we agree that HBoV probably has no relevance or primary role as a causative agent in LRTI in primary care (26). There may be an association between high HBoV viral loads and HBoV being the only virus detected (27), suggesting a quantitative approach should be considered (26).

This also applies to KIPyV and WUPyV. Although it is not yet possible to draw firm conclusions on their role in human pathology (28-30), our data show no evidence for a

causative role in outpatient CAP or LRTI: assessment of the viral loads could potentially help to further clarify their significance as well.

Limitations

Sputum was not obtained from all patients, and sputa and follow-up serology was not obtained from control patients. Consequently, a valid estimation of the prevalence of bacterial pathogens in controls was not always possible. Although the most important elements of this study are the descriptive results, we also performed multiple statistical tests so the finding of statistical significance may reflect type I error. However this is much less likely when supporting prior work on aetiology (e.g. bacterial causes of CAP) or when the p-value is very small (e.g. the case-control comparisons of viral aetiology).

Conclusions and implications for future management of LRTI

This unique comprehensive prospective study using modern microbiological methods suggests that the traditional view of aetiology in CAP and outpatient LRTI should be revised. We have found that viral CAP and LRTI is also caused by HRV, HCoV and HMPV. Our high viral detection rates should also inform clinical decision making. Better diagnostics are needed to distinguish viral from bacterial CAP or LRTI at the point of care.

The current study provides microbiological evidence why antibiotics do not help patients with LRTI. Only approximately one in five LRTI patients have a bacterial pathogen isolated and so could conceivably benefit from antibiotic treatment. This evidence should support primary care clinicians' restrictive approach to antibiotic prescribing for LRTI. If they consider antibiotics are indeed indicated, the low resistance levels in *S. pneumoniae* and *H. influenzae* should support the prescription of narrow spectrum antibiotics.

PROCEEDINGS

Part of this work was presented at the ECCMID, Berlin, Germany, September 27-30, 2013 (S-557).

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

CONTRIBUTORS

The larger GRACE observational study was designed by CCB, TV, PL, SC and HG, and sampling protocols by MI, CL, KL and HG. MI, CL, PL, TV and HG supervised the day-to-day management at study sites. PCR and serological analysis were done by KL, AV, CL, KH, KZ, EC, FC and AVL. Data were analysed by MI, KL and CL. Statistical analysis was performed by SC. The manuscript was drafted by MI, KL, SC and HG, and was reviewed by all authors.

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The funding sources were not involved in the design, conduct, analysis and interpretation of the data, nor in the writing and decision to submit the paper.

Table 1. Age and gender of all patients with LRTI, LRTI with CAP, LRTI without CAP, and of matched controls

	LRTI (n=3104)	LRTI with CAP (n=141)*	LRTI without CAP (n=2960)*	Matched ** controls (n=2063)
Gender				
Males, n= (%)	1244 (40.0)	62 (44.0)	1182 (39.9)	820 (39.7)
Females, n= (%)	1860 (60.0)	79 (56.0)	1781 (60.1)	1243 (60.3)
Age				
Mean (SD)	49.8 (16.8)	53.9 (15.3)	49.4 (16.6)	49.5 (16.6)
Range	18-92	19-87	18-92	18-92
Above 65years, n= (%)	628 (20.2)	40 (28.4)	588 (19.8)	385 (18.7)

* Data missing for three patients.

** Matched for age (maximum five years of difference) and gender, and consulting the same GP office for any other reason than LRTI within the same two-week period.

Table 2. Organisms detected in patients with LRTI, LRTI with CAP and LRTI without CAP

Organisms	LRTI (n=3104)	LRTI with CAP (n=141)	LRTI without CAP (n=2963)	P-value
Bacteria	n (%)	n (%)	n (%)	
S. pneumoniae	172 (5.5)	13 (9.2)	159 (5.4)	**0.043**
H. influenzae	167 (5.4)	20 (14.2)	147 (5.0)	**<0.001**
M. pneumoniae	150 (4.8)	6 (4.3)	144 (4.9)	0.738
C. pneumoniae	165 (5.3)	7 (5.0)	158 (5.3)	0.843
B. pertussis	95 (3.1)	4 (2.8)	91 (3.1)	1.000*
L. pneumophila	6 (0.2)	1 (0.7)	5 (0.2)	0.236*
Any of the above bacteria	655 (21.1)	42 (29.8)	613 (20.7)	**0.010**
Viruses				
Rhinovirus	623 (20.1)	20 (14.2)	603 (20.4)	0.066
Influenza A/B	307 (9.9)	11 (7.8)	296 (10.0)	0.378
Coronavirus	231 (7.4)	6 (4.3)	225 (7.6)	0.134
Respiratory syncytial virus	144 (4.6)	4 (2.8)	140 (4.7)	0.289
Human metapneumovirus	138 (4.4)	9 (6.4)	129 (4.4)	0.264
Parainfluenzaviruses 1-4	81 (2.6)	4 (2.8)	77 (2.6)	0.786*
Human adenovirus	41 (1.3)	5 (3.5)	36 (1.2)	**0.037***
Polyomaviruses	69 (2.2)	2 (1.4)	69 (2.3)	0.769*
Bocavirus	18 (0.6)	0 (0.0)	18 (0.6)	1.000*
Any of the above viruses	1494 (48.1)	53 (37.6)	1441 (48.7)	**0.010**

* Fisher's Exact test

Table 3. Viruses detected in LRTI patients and their matched controls

	Patients with LRTI,			Matched controls	
Organism, n/total (%)	Day 1 (n=3104)*	Day 28-35 (n=2673)*	P-value†	(n=2063)*	P-value†
Rhinoviruses	623 (20.1)	113 (4.2)	<0.0001	72 (3.5)	<0.0001
Influenza A/B	307 (9.9)	11 (0.4)	<0.0001	7 (0.3)	<0.0001
Coronaviruses	231 (7.4)	71 (2.7)	<0.0001	29 (1.4)	<0.0001
Respiratory syncytial virus	144 (4.6)	13 (0.5)	<0.0001	10 (0.5)	<0.0001
Human metapneumovirus	138 (4.4)	7 (0.3)	<0.0001	3 (0.1)	<0.0001
Parainfluenzaviruses 1-4	81 (2.6)	13 (0.5)	<0.0001	7 (0.3)	<0.0001
Adenoviruses	41 (1.3)	42 (1.6)	0.328	23 (1.1)	0.831
Polyomavirus	69 (2.2)	82 (3.1)	0.017	52 (2.5)	0.060
Polyomavirus WU	44 (1.4)	54 (2.0)		36 (1.7)	
Polyomavirus KI	27 (0.9)	28 (1.0)		17 (0.8)	
Bocavirus	18 (0.6)	11 (0.4)	0.433	16 (0.8)	0.161

* Denominator varies per aetiological agent due to 'not tested' in max 0.6% on day 1 and 3.7% of samples on day 28-35 and missing data in controls.

† The Generalized Estimating Equations took clustering of Day 1 and Day 28-35 samples within the same patients and clustering of Day 1 samples of patients and their matched controls into account.

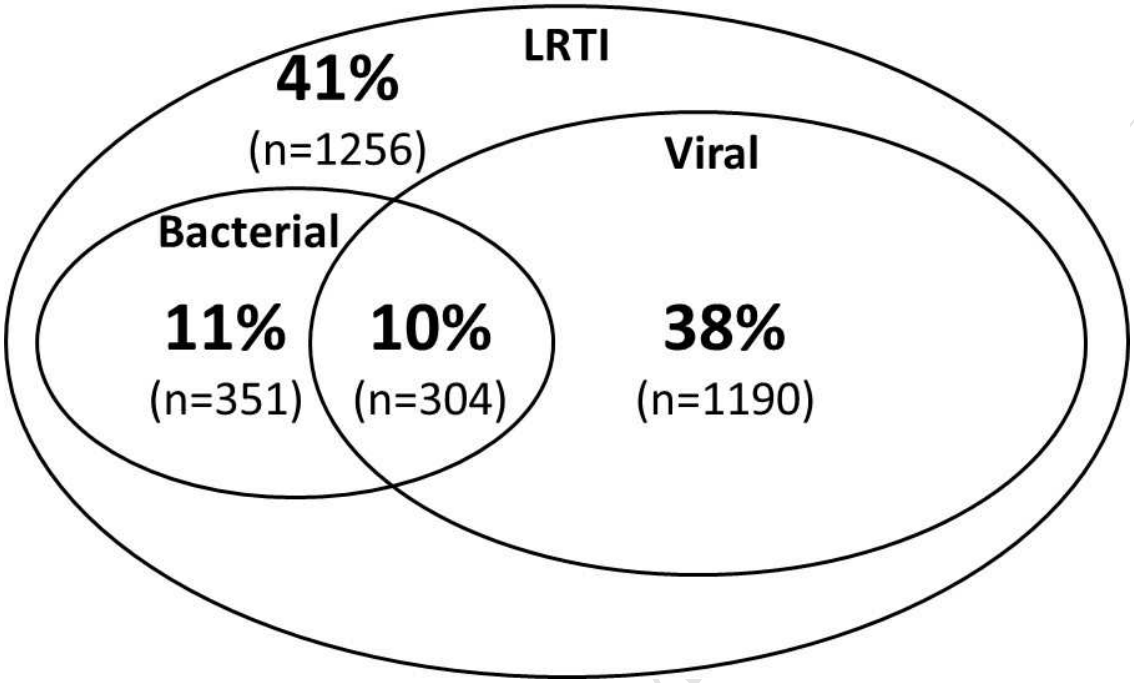
Table 4. Viruses and atypical bacteria detected at baseline (acute phase of illness) and at follow-up within the same patient

Virus or atypical detection by PCR	Baseline (acute illness) n (%) of total (n=3104)	At follow-up n (%) of positives during the acute phase
Bacteria		
M. pneumoniae	31 (1.0)	0 (0.0)
C. pneumoniae	26 (0.8)	1 (3.8)
B. pertussis	39 (1.3)	0 (0.0)
Viruses		
Rhinovirus	623 (20.1)	27 (4.3)
Influenza A/B	307 (9.9)	0 (0.0)
Coronaviruses	231 (7.4)	4 (1.7)
Respiratory syncytial virus	144 (4.6)	1 (0.7)
Human metapneumovirus	138 (4.4)	1 (0.7)
Parainfluenzaviruses 1-4	81 (2.6)	0 (0.0)
Adenoviruses	41 (1.3)	2 (4.9)
Polyomaviruses (WU+KI)	69 (2.2)	2 (2.9)
Bocavirus	18 (0.6)	1 (5.6)

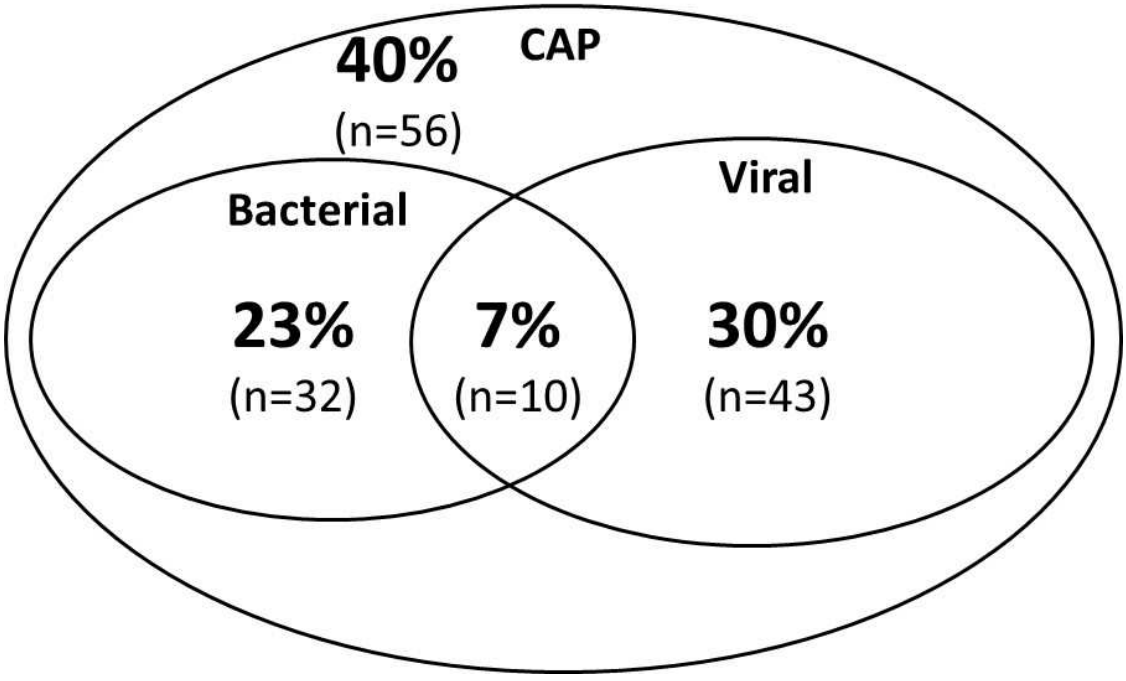
5

Figure 1. Venn diagrams of percentages (numbers) of patients with no, a bacterial, a viral or a mixed bacterial and viral aetiology detected in (a) 3104 patient with lower respiratory tract infections (LRTI) and in (b) 141 patients with community-acquired pneumonia (CAP) in primary care

a.



b.



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